UNITED STATES DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
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SAM - 314

9 CFR 113.47	June 1, 1985	Viruses Detected by FA
Standard Requirement	New	Agent

SUPPLEMENTAL ASSAY METHOD

FOR

DETECTION OF EXTRANEOUS VIRUSES BY

THE FLUORESCENT ANTIBODY TEST

A. SUMMARY

This standard assay method describes the fluorescent antibody staining procedure used by the Small Animal Biologics Virology Section to detect extraneous agents in master seed virus.

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B. MATERIALS

1. Cell cultures

Cell culture monolayers are grown on 8-chambered tissue culture slides.

2. Other materials

a. Conjugates

b. Dulbecco's phosphate buffered saline (PBS)

c. Deionized water

C. METHODS

Three groups of monolayers are stained. They are Group 1, the second passage of the master seed virus inoculated cell culture specified in 113.55(a); Group 2, the second passage of the uninoculated cell culture specified in 113.55(b); and Group 3, positive control monolayers derived from the master seed virus inoculated cell cultures at the time of the last subculturing [113.47(a)(1)(i)]. One positive control slide (8 monolayers) is made for each of the viruses specified in 113.47(b) by inoculating each monolayer with approximately 100 $TCID_{50}$ of the virus.

1. Staining procedure

a. At the time of staining, the plastic walls of the slide are removed, leaving the rubber gasket attached to the slide. The slides are rinsed in Dulbecco's PBS, fixed for at least 10 minutes in 4 C acetone, and dried with a hair dryer.

b. The slides are placed on a wet towel and approximately 0.1 ml of each conjugate is placed on each well of one slide from Groups 1

and 2 and the corresponding positive control slide from Group 3.

c. The slides are incubated at 37EC for 30 minutes, rinsed once in Dulbecco's PBS, and placed in a container of Dulbecco's PBS for 10 minutes.

d. The slides are rinsed thoroughly in deionized water and dried with a hair dryer.

2. Examination The slides are examined for fluorescence attributable to each specific virus. The 3 slides from each group with the same conjugate are compared. If the slide prepared from cells inoculated with master seed virus shows any evidence of specific viral fluorescence, the master seed virus is unsatisfactory.